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(71) Applicant *(for all designated States except US)*: **INTERCYTEX LIMITED** [GB/GB]; 48 Grafton street, Manchester M13 9XX (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LEEK, Michael, David** [GB/GB]; 57 Claydon Gardens, Rixton WA3 6FA (GB). **KEMP, Paul, David** [GB/GB]; Etherley Dene House, 16 Chadkirk Road, Romiley, Stockport, Cheshire SK6 3JY (GB).

(74) Agent: **DAVIES, Jonathan, Mark**; Reddie & Grose, 16 Theobalds Road, London WC1X 8PL (GB).

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(54) Title: WOUND HEALING USING FIBROBLASTS

(57) Abstract: The invention relates to scarring as a result of wound healing. In particular, the invention relates to a composition and a pharmaceutical composition comprising cells capable of reducing an inflammation response caused by wounding and a matrix-forming material, use of these compositions for reducing fibrosis and scarring, and a method of treatment of fibrosis and scarring during wound healing.

WOUND HEALING USING FIBROBLASTS

The invention relates to scarring as a result of wound healing. In particular, the invention relates to a composition, use of the composition and method of treatment of fibrosis and scarring during wound healing.

Wounds often heal with the production of a scar, which provides mechanical strength to a healed wound but can be unsightly. However, the exact mechanism of scar formation is not known. It has been proposed that it is likely to be a consequence of the body's need, in historical terms, to have a robust inflammatory response to any open wound in order to combat infection. The inflammatory response has the "side effect" of over stimulation of the synthesis of a fibre matrix (fibrosis). This inappropriate laying down of such fibres results in the appearance of a scar.

Fibrosis and the subsequent scarring as a result of wound healing is a serious clinical problem. Tissue that has healed inappropriately can lead to disfigurement, and the accompanying psychological effect on the person. Further, scarring can lead to an impairment function of the tissues. This is particularly important for organ tissues or other non-dermal tissues whose specialist function can be disrupted or impaired by scarring. Scarring as a result of surgery for tumour excision, for example.

Following wounding, the normal cellular healing response is triggered. This process involves a series of processes.

The initial phase concerns the formation of a blood clot with entrapped platelets. The platelets, in turn, release a mix of pro-inflammatory factors into the clot.

In the second phase, various factors released by the forming clot activate endothelial cells in the surrounding blood vessels, making them "sticky" to particular circulating white blood cells.

In the third phase, particular circulating blood cells become attached to the endothelium and then are attracted into the wound. The first of these cells (neutrophils) release cytotoxic substances to reduce infection. The second type of cells (monocyte/macrophage) then engulf the neutrophils and any cellular or matrix debris.

In a fourth phase, fibroblasts enter the wound from the surrounding tissues and begin the rebuilding process.

In a fifth phase, blood vessels grow into the wound. On some occasions the growth can be too vigorous and result in the reddening of the wound.

Although described here as five distinct phases, it will be appreciated that these phases are not always distinct, and that each phase may "blend" with the next.

It is, however, during the first and second phases when the triggers to inflammation and then the inflammatory factors themselves are released. It is during this time that it is thought that the triggers that cause fibrosis and scarring are released.

Currently, there is only one treatment approved and available to reduce scarring, that of silicone gel. However, the mode of action is unknown. Furthermore, the normal management of wounds is concerned with the initial closure of the wound (for example by sutures), and the prevention of infection and dessication of the wound with a suitable dressing. Little is done to prevent scarring in most cases,

although good standards of care can minimise fibrosis and scarring.

Surprisingly, it has now been found that the introduction of cells capable of reducing the inflammatory response caused by wounding reduces fibrosis and scarring. The cells are introduced at a wound site early in the healing cascade, and hence interfere with the subsequent phases of the cascade.

Further, it has been found that connective tissue cells are suitable cells. These include cell types such as fibroblasts, myoblasts or smooth muscle cells.

Thus according to the invention there is provided a composition comprising cells capable of reducing an inflammatory response caused by skin wounding and a cell delivery vehicle capable of delivering and maintaining the cells within a skin wound, wherein the cells are fibroblasts, the cell delivery vehicle is a matrix-forming material, and the composition is substantially free of other cell types, for use in the reduction of fibrosis and scar tissue during skin wound healing.

By "substantially free of other cell types", it is meant that the fibroblasts comprise at least 90%, preferably at least 91, 92, 93, 94, 95, 96, 97, 98 or 99%, of the cells. Alternatively, the composition may be completely free of cell types other than fibroblasts.

The composition at the time of incorporation of living cells may be free, or substantially free, of pre-formed matrix material. Matrix-forming material exists in a pre-matrix constitution in the composition but has the ability to form a scaffold or matrix around the cells in the composition. For example, if the composition is in the form of a gel, the matrix-forming material will solidify in use so that the

composition forms a construct comprising a scaffold or matrix containing the appropriate cells. This differs from prior art where cells are incorporated into an exiting, pre-formed matrix or scaffold. In the present invention, the scaffold or matrix may be formed around the cells either *in situ* or prior to application of the composition to the wound.

Thus the cells of the invention can be provided in a composition comprising a matrix-forming material that carries the cells to the wound site, and immobilises the cells at the site. Such matrix-forming material can be selected from fibrinogen/thrombin mixture, for example. The composition may be formulated with a suitable carrier to aid delivery to the wound site such as hyaluronic acid.

If a fibrinogen/thrombin mixture is used, at the wound site, thrombin converts fibrinogen to fibrin which forms a matrix, immobilising the cells at the required site. This has the advantage that the composition can be in a suitable form for storage prior to use. Further, the fibrinogen may be converted prior to administration or after administration to the wound site. In addition, when the composition is required it can be prepared using commonly known, pharmaceutically acceptable methods.

Further, fibrin may be used to form the matrix. This could be applied directly to the wound or in a suitable carrier which would deliver the composition to the wound site.

The composition of cells and matrix-forming material can be prepared in a pharmaceutically acceptable carrier, diluent or excipient prior to administration.

Alternatively, the composition can be formulated into a pharmaceutically acceptable form but then stored. When

required, the formulation can be made ready by the addition of a suitable diluent to form the required solution.

The fibroblasts may be mammalian, preferably human. The invention provides that the cells could be allogeneic cells, i.e. the cells administered to a patient would be from a donor.

Further provided according to the invention is a pharmaceutical composition comprising cells capable of reducing an inflammatory response caused by skin wounding and a cell delivery vehicle capable of delivering and maintaining the cells within a skin wound, wherein the cells are fibroblasts, the cell delivery vehicle is a matrix-forming material, and the composition is substantially free of other cell types, formulated with a pharmaceutically acceptable carrier, diluent or excipient.

The constituents of the pharmaceutical composition may include those as stated above for the composition.

The pharmaceutical composition as a formulation can be suitable for either topical delivery or parenteral delivery. For topical delivery, the formulation could be in the form of an ointment or paste for applying to the external surface of the wound. For parenteral delivery, the formulation could be in the form suitable for injection at the wound site. Such formulations would include solutions or suspensions, with or without a carrier such as a microsphere or microcapsule.

The pharmaceutical composition may be in the form of an ointment or paste for applying to the external surface of the wound.

Also provided is the use of a composition or a pharmaceutical composition according to the invention in the reduction of fibrosis and scarring as a result of skin wound healing.

In another aspect of the invention, there is provided the use of a composition or a pharmaceutical composition according to the invention in the manufacture of a medicament for the reduction of fibrosis and scarring during skin wound healing.

The invention encompasses a method of reducing fibrosis and scarring during skin wound healing comprising the administration to the patient of an effective amount of a composition or a pharmaceutical composition according to the invention.

It is envisaged that the composition or pharmaceutical composition of the invention may be used within a number of hours after a wound has formed, for example immediately after a wound has formed, although it may be preferable to administer it within a shorter time. Thus the composition or pharmaceutical composition may be used before the inflammatory phase of the skin wound. This has the effect of minimising fibrosis and scarring. The composition or pharmaceutical composition may be administered 2-48 hours, preferably 2-36 hours, after wounding.

Preferably the composition or pharmaceutical composition may be administered before inflammation of the wound site. In addition, it may be necessary to administer it over a sustained period of time.

The method is applicable when the skin wound is an acute skin wound or a chronic skin wound.

The invention also provides for a method of decreasing the wound healing time comprising administering an effective amount of a composition or pharmaceutical composition according to the invention.

Experimental example:

Porcine fibroblasts for the fibrin delivery vehicle (construct) were prepared as follows: a full thickness skin biopsy was taken and placed into a small volume of supplemented DMEM. Using fine forceps and scalpel the dermis was dissected away from fatty tissue and placed into a falcon tube containing 1xPBS. The biopsy was washed by shaking vigorously - this step was repeated 3 times. Following the washes, the biopsy was finely chopped using a scalpel and the resulting tissue transferred into a falcon tube containing a 1 x Collagenase solution (100U/ml). The tissue was continually agitated at 37°C for 90 minutes then allowed to settle. The supernatant was removed, placed into a fresh falcon tube and centrifuged at 1000rpm for 5 minutes. The pellet was re-suspended in 2ml of supplemented DMEM. A cell count was performed to assess number. Fresh collagenase solution was added to the digesting tissue and the above procedure repeated. The procedure was continued until the fraction yield diminished to approximately 10% of the highest fraction yield (typically 3-5 times). All fractions were combined and plated out at the desired density (10, 000 cells/cm^2). The cells were expanded and maintained until the required number achieved. The dissociation and expansion was typically performed 2 weeks prior to application of the construct to the porcine wounds.

Porcine fibroblasts were incorporated into the construct as follows: Each construct was cast with 500,000 cells in a final volume of 500ml. The desired number of cells were resuspended in 450ml of a 7.5mg/ml fibrinogen (typically purified from cryoprecipitate by methods known to those skilled in the art) and supplemented DMEM . In an alternative example, fibrinogen could be purchased from Sigma. Thrombin [Sigma: 50ml of (100U/ml)] was added to a 48 well plate, and shaken gently to ensure an even

distribution of thrombin over the base of the plate. The fibrinogen/cell suspension mixture was added to the thrombin and clotting occurred within seconds. 0.5ml of DMEM was added to the clot and allowed to mature for 24 hours.

Both excisional and incisional wounds (in Yorkshire pigs) made by standard methods to those skilled in the art were treated with the construct on Day 0 (day of wound) and day 4 (4 days post-wound). The wounds containing the constructs were covered by an occlusive dressing. Treatments were made in the following groups:

day 0: FB on fibrin matrix + epidermal graft (excisional)
FB on fibrin matrix - epidermal graft (excisional)
FB on fibrin matrix into an incisional wound

day 4: FB on fibrin delivery system + epidermal graft (excisional)
FB on fibrin delivery system - epidermal graft (excisional)
FB on fibrin matrix into an incisional wound

At day 4, 7, 21 and 56 wounds are macroscopically evaluated and punch biopsies are taken for histological evaluation. At 21 and 56 days after surgery wound contraction was determined.

Claims

1. A composition comprising cells capable of reducing an inflammatory response caused by skin wounding and a cell delivery vehicle capable of delivering and maintaining the cells within a skin wound, wherein the cells are fibroblasts, the cell delivery vehicle is a matrix-forming material, and the composition is substantially free of other cell types, for use in the reduction of fibrosis and scar tissue during skin wound healing.

2. The composition according to claim 1, wherein the fibroblasts comprise at least 90% of the cells.

3. The composition according to either one of claim 1 or claim 2, wherein the composition is free of cell types other than fibroblasts.

4. The composition according to any one of the preceding claims, wherein the composition is free of pre-formed matrix material.

5. The composition according to any one of the preceding claims, wherein the matrix-forming material is fibrinogen/thrombin.

6. The composition according to any one of the preceding claims, wherein the fibroblasts are mammalian, preferably human.

7. The composition according to any one of the preceding claims, wherein the fibroblasts are allogeneic.

8. The composition according to any one of the preceding claims, for use before the inflammatory phase of the skin wound.

9. A pharmaceutical composition comprising cells capable of reducing an inflammatory response caused by skin wounding and a cell delivery vehicle capable of delivering and maintaining the cells within a skin wound, wherein the cells are fibroblasts, the cell delivery vehicle is a matrix-forming material, and the composition is substantially free of other cell types, formulated with a pharmaceutically acceptable carrier, diluent or excipient.

10. The pharmaceutical composition according to claim 9, wherein the fibroblasts comprise at least 90% of the cells.

11. The pharmaceutical composition according to either one of claim 9 or claim 10, wherein the pharmaceutical composition is free of cell types other than fibroblasts.

12. The pharmaceutical composition according to any one of claims 9-11, wherein the pharmaceutical composition is free of pre-formed matrix material.

13. The pharmaceutical composition according to any one of claims 9-12, wherein the matrix-forming material is fibrinogen/thrombin.

14. The pharmaceutical composition according to any one of claims 9-13, wherein the fibroblasts are mammalian, preferably human.

15. The pharmaceutical composition according to any one of claims 9-14, wherein the fibroblasts are allogeneic.

16. The pharmaceutical composition according to any one of claims 9-15, in the form of an ointment or paste for applying to the external surface of the wound.

17. The pharmaceutical composition according to any one of claims 9-15, in the form of solution or suspension, optionally with a carrier such as a microsphere or microcapsule, for injection at a wound site.

18. The use of a composition according to claims 1-8 or a pharmaceutical composition according to claims 9-17 in the reduction of fibrosis and scarring as a result of skin wound healing.

19. The use of a composition according to claims 1-8 or a pharmaceutical composition according to claims 9-17 in the manufacture of a medicament for the reduction of fibrosis and scarring during skin wound healing.

20. A method of reducing fibrosis and scarring during skin wound healing comprising the administration to the patient of an effective amount of a composition according to claims 1-8 or a pharmaceutical composition according to claims 9-17.

21. The method according to claim 20, wherein the composition or pharmaceutical composition is administered immediately after wounding.

22. The method according to claim 21, wherein the composition or pharmaceutical composition is administered 2-48 hours, preferably 2-36 hours, after wounding.

23. The method according to any one of claims 20-22, wherein the skin wound is an acute skin wound.

24. The method according to any one of claims 20-23, wherein the skin wound is a chronic skin wound.

INTERNATIONAL SEARCH REPORT

Inte ial Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	RU 2 023 424 C (N PROIZV TS TRANSPLANTATSII I) 30 November 1994 (1994-11-30) Abstract --- -/--	1-4,6,7, 9-12, 14-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Beranová, P

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